JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 905–916

Received December 11, 2006
Accepted March 12, 2007
DOI 10.1211/jpp.59.7.0001
ISSN 0022-3573

EXHIBIT C

Review Article

Prediction of human pharmacokinetics – gastrointestinal absorption

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Abstract

Permeability (P_e) and solubility/dissolution are two major determinants of gastrointestinal (GI) drug absorption. Good prediction of these is crucial for predicting doses, exposures and potential interactions, and for selecting appropriate candidate drugs. The main objective was to evaluate screening methods for prediction of GI P_e, solubility/dissolution and fraction absorbed (f_a) in humans. The most accurate P_e models for prediction of f_a of passively transported and highly soluble compounds appear to be the 2/4/A1 rat small intestinal cell model (in-vitro and in-silico), a newly developed artificial-membrane method, and a semi-empirical approach based on in-vitro membrane affinity to immobilized lipid bilayers, effective molecular weight and physiological GI variables. The predictability of in-vitro Caco-2, in-situ perfusion and other artificial membrane methods seems comparably low. The P_e and f_a in humans for compounds that undergo mainly active transport were predicted poorly by all models investigated. However, the rat in-situ perfusion model appears useful for prediction of active uptake potential (complete active uptake is generally well predicted), and Caco-2 cells are useful for studying bidirectional active transport, respectively. Human intestinal in-vitro P_e, which correlates well with f_a for passively transported compounds, could possibly also have potential to improve/enable predictions of f_a for actively transported substances. Molecular descriptor data could give an indication of the passive absorption potential. The 'maximum absorbable dose' and 'dose number' approaches, and solubility/dissolution data obtained in aqueous media, appear to underestimate in-vivo dissolution to a considerable extent. Predictions of in-vivo dissolution should preferably be done from in-vitro dissolution data obtained using either real or validated simulated GI fluids.

Introduction

Background

The requirement for good prediction of gastrointestinal absorption Thummel et al (1997) investigated the impact of low oral bioavailability (F) on the variability of systemic exposure for 143 substances, and found a decreasing coefficient of variance (CV) with increasing F. Average (range) estimates of CV for drugs with F values of 0–0.20 and 0.80–1.00 were 40–50% (10–100) and 15–20% (5–35), respectively. Potential benefits of high F, other than less inter- and intra-individual variation in systemic exposure, include smaller dosage forms and lower material costs. Thus, it is desirable to find candidate drugs (CDs) with sufficiently high F. A prerequisite for this is that accurate methods for prediction of the gastrointestinal (GI) fraction absorbed (f_a) are available and applied. In order to find and select suitable CDs and to reject those with unfavourable absorption characteristics, the factors that determine GI absorption also need to be well understood and considered.

Good prediction of absorption is also required for correct classification according to the Biopharmaceutics Classification System (BCS). The BCS is a regulatory tool that was developed to enable replacement of in-vivo bioequivalence studies for immediate-release products by permeability (P_e) and in-vitro dissolution tests (FDA Guidance for Industry 2000). A recent evaluation shows that the BCS has a strict solubility/dissolution limit, a generous P_e limit (limit for rate constant for dissolution is ≥ 14 times higher than that for permeation), and is stricter for compounds with long half-life ($t_{1/2}$) (Fagerholm 2007a). The potential implication of these findings is that many true BCS class I drug products

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Acknowledgements: My colleagues at AstraZeneca R&D Södertälje for inspiration, and Associate Professor Anna-Lena Ungell for reviewing and giving valuable input to the manuscript.

This paper includes personal opinions of the author, which do not necessarily represent the views or policies of AstraZeneca.

(high P_e and high solubility) are classified incorrectly. This could possibly explain the limited use of this system. In order to obtain a better balance and to enable an increase in the number of biowaivers, it has been suggested that the limit for high solubility/dissolution be decreased, the limit for high P_e be increased, the $t_{1/2}$ be considered, and that the rate-limiting step for in-vivo absorption be determined. Another major proposal was to reduce the BCS into two classes: permeation-rate (class I) or dissolution-rate (class II) limited absorption.

Determinants for gastrointestinal absorption The major determinants for f_a are GI P_e, solubility/dissolution, absorption time and stability (Dressmann et al 1985; Fagerholm 1997; van de Waterbeemd & Jones 2003). The P_e is defined as the speed (cm s^{-1}; typically 10^{-4} or 10^{-6} cm s^{-1}) at which a molecule is transported (by passive diffusion and/or active transport) across a membrane, cell, endothelium or epithelium. This parameter is determined by the interplay between the characteristics of the membrane, cell, endothelium or epithelium and the molecules. Molecular properties that are important for the permeation include molecular weight (MW), size, shape, conformation, degree of ionization, polar surface area (PSA), non-polar surface area, charge, lipophilicity and number of H-bonding acceptors and donors (Palm et al 1997, 1999; Stenberg et al 1999; Lipinski et al 2001). The MW and lipophilicity of CDs appear to have increased over time, and this has led to poorer intestinal P_e and/or solubility (Lipinski 2000). According to Lipinski, poor GI solubility is now the largest problem preventing good oral absorption and F (Lipinski 2005). On this basis, there are now higher demands on methods for prediction of P_e, solubility/dissolution and f_a.

Membranes on the basolateral (blood) side of intestinal mucosal cells are thinner, and contain less cholesterol and glycolipids than apical (GI lumen side) membranes (Kararli 1995). This makes them more fluid and permeable than apical membranes. The sets of drug-transporter proteins in these membranes also differ. For example, P-gp, BCRP and MRP2 are efflux proteins in the apical membrane, MRP3 is an influx transporter on the basolateral side, and PEPT1 and ASBT are influx transporters on the apical side of human intestinal mucosal cells (Sugiyama et al 2006). Mucosal cells from the small and large intestines (enterocytes and colonocytes) also differ with regard to membrane composition and sets and expression of transporter proteins (Kararli 1995; Ungell et al 1998; Englund et al 2006; Seithel et al 2006). Another difference is that the small intestinal mucosa has villi structures that greatly increase its surface area for absorption (Kararli 1995; Fagerholm 1997). In-vitro data obtained using the Ussing chamber technique show the following passive P_e-characteristics of the human intestine: duodenum>jejunum>ileum≥colon for hydrophilic compounds, and duodenum<jejunum<ileum≤colon (large intestine) for lipophilic compounds (Sjöberg et al 2000). In contrast to the small intestine, the colon has a low capacity to actively absorb nutrients (Fagerholm et al 1997; Ungell et al 1998; Sjöberg et al 2000). This indicates low/absent colonic expression of nutrient transporter proteins. Seithel et al (2006) compared mRNA expression of several important drug transporters in human enterocytes and colonocytes, and found that colonocytes have much lower levels. Englund et al (2006), however, demonstrated that many transporters are expressed at similar or higher levels in the colon. For example, the expression of the well-known P-pg was 5 times higher in the human ileum than in the duodenum and colon, and OCT1, MRP3, OCTN2 and MCT1 showed highest expression in the colon. Paracellular (transport between mucosal cells) drug absorption and diffusion limitations through the unstirred water layer (UWL) adjacent to the GI mucosa do not appear to be of any significant importance in-vivo (Fagerholm et al 1995, 1999; Fagerholm and Lennernäs 1995).

Factors that are important for drug solubility/dissolution include pK_a, surface area, diffusivity, lipophilicity, molecular size, crystalline energy and P_e of the compound, particle size and wettability, and pH, surface tension, composition, solubilization, buffer capacity, viscosity, mixing and volume of GI fluids (Dressmann et al 1998; Hörter and Dressmann 2001; Kostewicz et al 2002). The average fasted-state pH in the human stomach, duodenum, jejunum and ileum has been reported to be 1.3, 6.5, 6.6 and 7.4, respectively (Dressmann et al 1998; Hörter and Dressmann 2001). Consequently, weak acids have lower solubility in the stomach than in the intestines, and the opposite occurs for weak bases. Other reported values for proximal small intestinal pH in the fasted state are 5.1 ± 0.6, 6.6 ± 0.5 and 7.1 ± 0.6 (Lindahl et al 1997). The pH falls below 6 at the entry into the colon, and is then raised to ~7 in the distal parts (Abrahamsson 1997). The pH in the fluid adjacent to the enterocytes in-vivo is one unit lower than in the GI lumen (Rawlings et al 1987). Changes in pH along the GI tract could affect solubility or precipitation of dissolved drug. Solubility is one of the determinants for the rate and extent of dissolution; thus, dissolution is a more relevant parameter than solubility for prediction of in-vivo GI absorption (Rinaki et al 2003; Yu et al 2004; Lennernäs and Abrahamsson 2005). P_e is also a determinant of in-vivo solubility/dissolution. A high P_e provides sink conditions, which will favour the extent and rate of in-vivo dissolution. Compounds with low GI solubility and P_e will not or may not reach significant sink conditions and must rely on dissolution in more distal parts of the GI tract where the fluid has higher viscosity, contains lower amounts of solubilization agents (such as bile salts) and is less well mixed.

The time available for mucosal drug uptake depends on the GI transit time (TT), convection/mixing, radius and viscosity of the GI contents (Fagerholm et al 1996). The gastric residence time is short in relation to intestinal TT, especially for fluid and during fasting. Depending on the activity phase of the GI tract, the gastric emptying $t_{1/2}$ and lag-time of 200 mL fluid are 5–23 min and 2–16 min, respectively (Dressmann et al 1998). In another study, the gastric emptying rate constant (k_{ge}) for solutions was estimated to be 3.8 h^{-1} ($t_{1/2}$ ~10 min; 90% emptied within ~15 min) (Adkin et al 1995). For particles of 1 mm and greater, and after intake of a meal, the gastric residence time could be more than 1 h (Dressmann et al 1998). The uptake of drugs by the gastric mucosa is also limited by a comparably low P_e. The TT in the small and large intestines are reported to be 3 ± 1 and 36 h (range 1 to > 60) h, respectively (Davis 1986). Compounds not

completely absorbed by the small intestine, which is the main site for absorption of most drugs and nutrients, will enter the large intestine. In this region, the luminal contents are more viscous and less well mixed than in the stomach and small intestine, and the diameter is greater than that of the small intestine (Fagerholm 1997). These features make it difficult to predict the time available for uptake (at the mucosal surface) and the extent of absorption from the colon.

Some compounds, for example, esters and peptides, are degraded by enzymes or microorganisms (mainly in the distal ileum and colon) in GI fluids (Kararli 1995; Krishnamoorthy and Mitra 1995; Fagerholm et al 2005; Fagerholm and Björnsson 2005). In general, most drugs are stable within the GI fluids.

Species similarities and differences An understanding of the similarities and differences in absorption capacity and GI physiology between species is also helpful when selecting CDs and predicting the f_a in humans. Intestinal mucosal membranes are comparable across species (Chiou and Buehler 2002), but sets, expression and activities of drug-transporter proteins could differ (Fagerholm et al 1996; Chiou and Barve 1998; Sugiyama et al 2006). Species also differ in expression levels and patterns of intestinal metabolizing enzymes, microorganisms and flows and concentrations of intestinal secretions (including bile) (Davies and Morris 1993; Kararli 1995; Cao et al 2006). Compared with humans, rats have shorter small intestinal length (0.8 vs 7 m), radius (0.18 vs 1.75 cm) and TT (1.5 vs 3 h), similar intestinal pH and total bile salt concentration, and higher bile flow rate (90 vs 5 mL day^{-1} kg^{-1}) and intestinal β-glucuronidase activity (Davies and Morris 1993; Kararli 1995; Chiou and Barve 1998). Compared with humans, dogs have shorter small intestinal TT (2 vs 3 h), higher bile flow rate (12 vs 5 mL day^{-1} kg^{-1}), longer villi (and therefore larger absorptive area), higher bile acid and salt concentrations, and similar intestinal pH (Davies and Morris 1993; Kararli 1995; Lennernäs 1997; Chiou and Barve 1998). The intestinal pH in monkeys is reported to be similar to or slightly lower than in man, while the bile flow rate is higher (25 vs 5 mL day^{-1} kg^{-1}) (Davies and Morris 1993; Chiou and Buehler 2002).

Objective

The main objective was to evaluate screening methods for prediction of GI P_e, solubility/dissolution and f_a in humans, and to determine whether the predictability is sufficiently good for correct stop–go decisions, and safe and effective dosing in early clinical studies. Predictions of absorption interactions (such as the impact of food on P_e, solubility/dissolution and f_a) were not evaluated.

First, predictions of intestinal absorption from animals to human are presented and analysed. Then, models for prediction based on P_e and molecular descriptors for prediction of f_a are evaluated. Thereafter, methods for prediction of f_a for compounds with low solubility are investigated. Finally, the performance of a new approach based on the average P_e obtained in the small intestine (2/4/A1 model) and large intestine (Caco-2 model) is evaluated.

Methods for prediction of gastrointestinal absorption

Prediction of fraction absorbed in humans from fraction absorbed and oral bioavailability in animals

A high correlation between f_a values in rats and humans was shown by Chiou and Barve (1998). The $f_{a,man} = 0.99 \times f_{a,rat}$ ($r^2 = 0.97$) for 64 test substances, and $f_{a,man} = 0.91 \times f_{a,rat} + 0.02$ ($r^2 = 0.90$) for the 24 substances with $f_a < 0.9$ (Chiou and Barve 1998). The compounds varied widely in their physicochemical properties. Solubility/dissolution-limited absorption was not fully investigated, and since animals were given the compounds as solutions or suspensions and humans were given tables/capsules, this could possibly have influenced the results.

Zhao et al (2003) compared f_a data for 98 substances in humans and rats. Compounds with suspected dissolution-rate-limited absorption were not included in the set, but those with active transport were. The obtained relationship ($f_{a,man} = 0.997 \times f_{a,rat}$; $r^2 = 0.88$) was similar to that shown by Chiou and Barve (1998) but, as expected on the basis of inclusion of actively transported substances, the prediction errors were larger.

Chiou et al (2000) compared f_a data in humans and dogs for 43 compounds (solutions, suspensions, tablets and/or capsules), and found a poor relationship; absorption was generally better in the dog. This trend was further demonstrated with different polyethylene glycols (PEGs) by Chiou and Barve (1998). These features and findings show that the dog is a not the most suitable animal model for prediction of oral drug absorption in man.

Monkeys and pigs are also commonly used in drug development. F_a data in pigs are rare, and a direct comparison of f_a data in humans and pigs for a sufficient number of substances has not been demonstrated/published. Available f_a data from monkeys (rhesus and cynomolgus macaques) (solutions, suspensions, tablets and/or capsules; 43 compounds) showed a strong linear relationship with those in humans ($f_{a,monkey} = 0.96 \times f_{a,man} + 0.03$; $r^2 = 0.97$) (Chiou and Buehler 2002). Most substances with incomplete uptake had been given as tablets to humans, but as solutions to monkeys. Five substances with incomplete absorption were administered in similar dosage forms to the two species, and the f_a data for these were similar in humans and monkeys.

On the basis of these observations, the rat and monkey seem to be good model species for prediction of human f_a, and especially when the uptake is mainly passive. A limitation with this approach is that it is not suitable for high throughput screening. It should be noted that the absorption rate and absorption rate constant (k_a) are expected to differ between animals and humans because of the influence of intestinal radius and gastric emptying time. The difference in intestinal radius between humans and rats (10 fold) is greater than the difference in intestinal passive P_e (on average 3.6 fold) (Fagerholm et al 1996), and gastric emptying is slower in humans. Absorption is therefore expected to be slower in humans than in rats and other laboratory animals.

Sietsema (1989) and Cao et al (2006) showed that there are virtually no relationships between species with regard to

oral F, which indicates the limitations of animal data for prediction of F in man. This can be explained by considerable species differences in gut-wall and hepatic clearances in addition to differences in f_a (especially between dogs and humans) (Bogaards et al 2000; Clarke and Jeffrey 2001; Ward and Smith 2004; Cao et al 2006). This suggests that in order to predict F well, each of the processes determining F (f_a and gut wall and hepatic extraction) need to be predicted separately. Animal F data could still be useful for predictions. Based on the species similarities in f_a, F data obtained in rats and monkeys could indicate the minimum f_a in man (at least when uptake is mainly passive).

Prediction of fraction absorbed in humans from permeability data and molecular properties

The approaches presented can be applied to compounds that are anticipated to have good solubility/dissolution in-vivo, and with pK_a outside the critical range (~5–7; intestinal pH range). The potential impact of changes in pH on the degree of ionization and P_e has been demonstrated by Palm et al (1999), and Neuhoff et al (2003, 2005). Palm et al (1999) studied differences in the P_e of the ionized and unionized forms of two weak bases in Caco-2 cells, and found that P_e values for the unionized forms of alfentanil (pK_a 6.5, high P_e) and cimetidine (pK_a 6.8, moderate P_e) were 150- and 30-fold higher, respectively, than for the ionized forms.

In-situ perfusion Passive small intestinal P_e values in rats and humans correlate well, at least when the P_e is moderate or high (Fagerholm et al 1996; Cao et al 2006). Cao et al (2006) also showed that rats and humans have similar patterns of expression of small intestinal transporters and drug absorption profiles for compounds that undergo active transport (quite high correlation between rat and human in-situ perfusion P_e). Based on this finding, they concluded that perfusion P_e data from rats can be used to predict drug absorption from the human small intestine following oral dosing. However, most of the compounds studied had high active P_e (predicts complete f_a), and perfusion P_e in rat and man (Loc-I-Gut technique) is a poor predictor of f_a (unless $f_a \sim 1$). (Perfusion methods are not sensitive enough to measure the P_e of compounds with low or moderate P_e; see Fagerholm et al (1996); Kasim et al (2004); Fagerholm (2007a). Furthermore, the mean and maximum P_e prediction errors were 2–3- and 10-fold, respectively. Therefore, it appears that the rat perfusion model is mainly useful for predicting the active uptake potential (complete active uptake was well predicted). Another limitation with this approach is the comparably slow screening rate.

Caco-2 cell line The Caco-2 cell model is commonly used for screening/estimation of P_e and prediction of human f_a (Artursson et al 2001). These cells are of colonic origin but, unlike normal colonocytes, they express similar drug transporters to the human small intestine (Seithel et al 2006). Studies of Caco-2 have demonstrated the activity of these transporters (Neuhoff et al 2003, 2005; Englund et al 2006). Englund et al (2006) showed that the expression of drug transporters in Caco-2 was closer to that of the small intestine than to that of the colon. Seithel et al (2006) reported that the expression of most well-known drug transporters is lower in Caco-2 cells than in human enterocytes. Differences in the composition of cell membrane, paracellular radius and transporter expression imply that the uptake characteristics of Caco-2 cells are different from that of the small intestine (Kararli 1995; Ungell et al 1998; Balimane and Chong 2005). Thus, these cells seem useful for studying active intestinal transport rather than for predicting f_a for actively transported compounds. The correlation between values for P_e obtained from Caco-2 cells in-vitro and from human small intestine in-vivo, and predictability of human f_a in many studies, are comparably weak, particularly when active transport is involved (Lennernäs et al 1996; Yazdanian et al 1998; Irvine et al 1999; Salphati et al 2001; Grass and Sinko 2002; Parrott and Lavé 2002; Sun et al 2004). Irvine et al (1999) compared Caco-2 P_e (at pH 7.4) and human f_a data for about 40 passively absorbed compounds, and found a poor correlation between the two parameters. For example: for two passively absorbed compounds with an f_a of 0.75, P_e varied by more than four orders of magnitude; f_a could not be predicted accurately for P_e values between ~0.1 and $\sim 1 \times 10^{-6}$ cm s^{-1} (f_a range ~0.05 to ~0.7); compounds with P_e values of $\sim 2 \times 10^{-6}$ cm s^{-1} had f_a values ranging from ~0.2 to 1.0; a scattered picture was found for the numerous compounds with f_a values between 0.8 and 1.0. The Caco-2 P_e data could not predict well the f_a for compounds with low P_e and could not be used to classify compounds with moderate-to-high P_e according to the limits of the BCS. Similar results were demonstrated by Grass and Sinko (2002) and Parrott and Lavé (2002). Parrott and Lavé (2002) used Caco-2 P_e and solubility data and IDEA and GastroPlus software to predict the human f_a of 28 drugs. They found rather scattered pictures and a trend towards overestimation of f_a (especially at low f_a) with both models. The data set included compounds with active uptake and efflux. With the IDEA software, 39% of predictions were overestimations (defined as more than 10% relative error) and 21% were underestimations. The maximum error was +260% (predicted $f_a = 0.96$, observed $f_a = 0.27$). Corresponding values for the GastroPlus software were 36% overestimations, 21% underestimations and +500% maximum error (predicted $f_a = 0.18$, observed $f_a = 0.03$), respectively. About one-third of compounds with an f_a of 0.5 or more were incorrectly P_e classified (BCS) with both programmes. The relatively poor predictability of the Caco-2 cell model is probably the main reason for the poor performance. It does not seem that any of the test compounds had solubility/dissolution-limited uptake. Only one compound (verapamil) had a dose number (D_o) exceeding 100 ($D_o = 143$), and this compound is completely absorbed in-vivo. (This parameter is discussed in more detail below.) Matsson et al (2005) showed the relationship between Caco-2 P_e values (apical to basolateral direction; pH 7.4) and f_a for 14 compounds with mainly passive absorption. With this comparably small number of compounds, the predictability of the Caco-2 model appeared to be quite good (Figure 1) and was better than that demonstrated by Irvine et al (1999), Salphati et al (2001), Grass and Sinko (2002) and Parrott and Lavé (2002). Thus, the Caco-2 model generally works well to predict complete or near complete f_a for highly permeable substances.

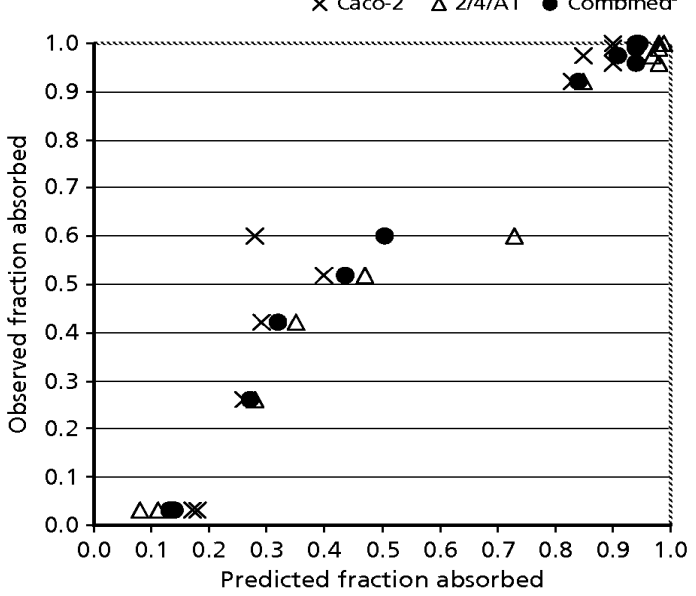

Figure 1 Predicted vs observed fraction absorbed (f_a) with the Caco-2 and 2/4/A1 cell models, and the average (combined) of these two models. Predicted and observed data for 12 compounds with mainly passive absorption (alfentanil, alprenolol, antipyrine, atenolol, clodronic acid, didanosine, mannitol, metoprolol, olsalazine, pindolol, propranolol and terbutaline) were approximated or extracted from Matsson et al (2005). Creatinine was excluded from the data set because its f_a value could not be found in the referred literature, and lactulose was excluded because of its gastrointestinal instability (Meddings 1997). The observed f_a for terbutaline was set to 0.60, which is the average value used in many reports (Borgström et al 1989; Chiou and Barve 1998; Irvine et al 1999; Willmann et al 2004).

The Madin–Darby canine kidney (MDCK) model The MDCK model has also been used to estimate P_e data and to predict human f_a. Because of its origin, active transport across these cells differs from that of human intestinal cells (Balimane and Chong 2005). It is also likely that these cells have a different membrane composition from intestinal cells. Irvine et al (1999) related MDCK P_e values (at pH 7.4) and human f_a values for ~40 passively absorbed compounds, and found that the accuracy and precisions were similar to that of the Caco-2 cell line. A similar result was shown for fewer substances by Salphati et al (2001). Thus, this model is of limited applicability for prediction of f_a, at least for compounds with low or moderate P_e.

The 2/4/A1 cell line In-vitro P_e data obtained with 16 passively absorbed compounds in the rat small intestinal cell line 2/4/A1 (pH 7.4) were shown to predict the human f_a better than the Caco-2 model (Matsson et al 2005; Tavelin et al 2003a). Five substances with f_a values ranging from 0.96 to 1.00 were correctly classified to have high P_e (in-vivo $f_a > 0.9$), whereas one compound with $f_a = 0.92$ was predicted to have f_a ~0.85 (incorrect BCS classification). Caco-2 data predicted f_a to be 0.83–0.90 for these compounds. In contrast to the Caco-2 cells, the 2/4/A1 cells lack expression of several drug transporters and they generate passive P_e values comparable to those for the human intestine. The drawbacks with this model, other than the lack of important transporter proteins, are that small temperature changes may introduce variability, and cells grown at 37°C are more poorly differentiated than the fraction that survives at 39°C (Tavelin 2003b). In addition, only a limited amount of data are available. Further experiments are needed to fully evaluate the potential of this method. As shown below, an in-silico prediction method based on these data seems quite good.

Artificial membranes Artificial membranes, which lack transporter proteins and a paracellular pathway, have also been used for P_e screening and prediction of f_a. In the study by Matsson et al (2005), P_e data obtained with artificial hexadecane membranes correlated with f_a data although the correlation was poorer than for the Caco-2 and 2/4/A1-models, demonstrating a limited usefulness of this approach.

Flaten et al (2006) compared the predictability of a new phospholipid-vesicle-based permeability model with PSA, log D, immobilized liposome chromatography (ILC), parallel artificial membrane permeation assay (PAMPA), double sink PAMPA and Caco-2 models. The dataset included 21 drugs, including compounds that undergo active transport. The phospholipid-vesicle-based model seems to model the in-vivo absorption better than PSA, log D, ILC, PAMPA, and equally well as Caco-2 and double sink PAMPA models.

Corti et al (2006) have developed and evaluated a new in-vitro permeation method based on artificial membranes. The P_e values for a set of 21 different drugs (similar set to that used by Flaten et al 2006) were measured. The absolute prediction errors were within 5–10%, and the predictive ability was better than with PAMPA and Caco-2 cells. An interesting finding was that the relationship between P_e in the artificial membranes and in-vivo f_a was linear, which is not normally seen with permeation methods. The good predictability of f_a for mainly passively transported compounds makes this an attractive approach.

Willmann et al (2004) developed a semi-empirical approach, in which membrane affinity to immobilized lipid bilayers and effective MW (correction of the MW for molecules with halogen atoms) were used together with physiological GI variables (r, TT and pH) to predict the f_a in man for 126 substances. The ionized forms were assumed to be 1000 times less permeable than the unionized forms (which is greater than suggested by data by Palm et al (1999); see above). They found good predictions of f_a for substances with P_e-limited uptake and mainly passive P_e (unity slope; $r = 0.97$), whereas the f_a of compounds with pronounced active uptake and efflux was poorly predicted. F_a values for passively transported substances with low f_a could be well predicted. The prediction errors for compounds with f_a values above 0.2 were maximally ~20–30% (relative %). Predicted f_a values for compounds with actual f_a of ~ 0.1, ~ 0.2 and ~ 0.9 ranged between ~ 0.05 and ~ 0.13 ($n = 2$), ~ 0.15 and ~ 0.28 ($n = 3$), and ~ 0.75 and 1.0 ($n > 10$), respectively. The apparently good correlation between predicted and observed f_a for compounds with low, moderate and high f_a, together with the possibility of a high screening rate, demonstrates the potential of this approach for absorption screening and prediction. The scattered picture for f_a values between 0.8 and 1.0 shows that the model does not allow accurate BCS P_e classification for compounds with near-complete or complete

absorption, but it is sufficiently good to predict complete or near complete f_a for substances with very high P_e.

In-silico models Several attempts have been made to study and establish relationships between different molecular properties and P_e or f_a. For example, trends of decreasing P_e with increasing MW, and increasing P_e with increased lipophilicity (up to a certain level) have been shown (Ungell et al 1998; Artursson et al 2001). Palm et al (1997) demonstrated a sigmoidal relationship between PSA and f_a in man for 20 structurally diverse compounds, and the data suggested that the f_a in man could be predicted well from PSA data. A drawback with the PSA approach is that it cannot account for lipophilic effects introduced by the addition of non-polar substituents. Later, Stenberg et al (1999) demonstrated the importance of non-polar surface area for a series of oligopeptide derivatives. For larger data sets (> 100 compounds) there was a rather poor correlation between PSA and human f_a (Clark 1999; Willmann et al 2004). Clark (1999) found that the criterion of PSA greater than 140 $Å^2$ is a reliable predictor of poor absorption. Only a few compounds with a PSA above this value have moderate or high f_a (Clark 1999; Willmann et al 2004). This approach seems more reliable than Lipinski's empirical 'rule-of-five', which states that the GI absorption is potentially low if at least two of the following criteria are met: MW above 500 g $mole^{-1}$; more than 10 hydrogen-bond acceptors; more than five hydrogen-bond donors: log P greater than 5.0 (Lipinski et al 2001). About 10% of 2245 compounds in the World Drug Index had values above each of these limits (Clark & Grootenhuis 2003). A modified rule (for a series of p38 MAP kinase inhibitors) was presented by Clark and Grootenhuis (2003). According to their rule, the f_a is potentially low if PSA is greater than 140 $Å^2$ and at least two of the following criteria are met: MW greater than 550 g $mole^{-1}$; more than 12 hydrogen-bond acceptors; more than five hydrogen-bond donors; log P above 6.2.

More sophisticated approaches to predict the human f_a from molecular descriptors exist (Klopman et al 2002; Clark & Grootenhuis 2003; Bergström 2005; Matsson et al 2005). Matsson et al (2005) developed in-silico models for prediction of human f_a based on two- and three-dimensional molecular descriptors and Caco-2 or 2/4/A1-P_e data. They found that a 2/4/A1-based in-silico model gave results of similar quality to those obtained using experimentally derived 2/4/A1 P_e data, and better than for the artificial hexadecane membrane model (see above). Based on the comparably good predictability of 2/4/A1 cell P_e data, and the ability to find a computational method that could produce similar predictions, this approach appears highly attractive. However, the success of in-silico models depends on the quality of P_e and f_a data that are used in the modelling. The uncertainty of f_a data could also be a reason for poor predictability of such a model.

Human intestinal mucosa (Ussing chamber) Obradovic (2005) found that P_e values obtained for human small intestine in-vitro (Ussing chamber) for a large set of structurally diverse passively absorbed compounds correlated well with in-vivo f_a values (the figure has not yet been published). This approach could potentially also be useful for prediction of f_a for compounds that undergo active transport. It should be borne in mind, however, that metabolism within intestinal cells could influence in-vitro estimates of P_e. There is potential for underprediction of P_e for compounds with extensive gut-wall metabolism, such as substrates of CYP3A4 and conjugating enzymes.

Prediction of fraction absorbed in humans from dissolution and permeation data

Prediction of f_a for slowly dissolving and incompletely dissolved substances is more difficult than for compounds with P_e-limited uptake. For such substances, both P_e and solubility/dissolution need to be incorporated in a prediction model. Thus, it is important to know when solubility/dissolution could be the, or a, rate-limiting step for absorption. Wu and Benet (2005) showed that 41–44 of 131 commonly prescribed drugs (31–34%) belong to BCS class II (high P_e, low solubility; based on in-vitro solubility/dissolution), and that a small fraction of drugs belong to BCS class IV (low P_e, low solubility). This indicates that limited GI solubility/dissolution is common. In order to evaluate whether this is actually true, D_o data for highly permeable substances with high D_o (> 10 in aqueous media or simulated intestinal fluid (SIF) at physiological pH) were collected or calculated and correlated with available f_a data (73 compounds). The dimensionless D_o is a parameter that is commonly used as a measurement of dissolution potential. It is defined as the ratio of dose concentration to solubility; D_o = (highest dose strength / 250 mL fluid) / solubility (Dressmann et al 1985). A D_o value of 1 implies that the expected highest GI concentration is similar to the solubility; a high D_o implies low dissolution potential. Data for D_o vs f_a for these 73 high-P_e compounds (Figure 2) clearly demonstrate the poor relationship between in-vitro solubility and in-vivo dissolution (Irvine et al 1999; Kataoka et al 2003; Zhao et al 2003; Kasim et al 2004; Peréz et al 2004; Willmann et al 2004; Yazdanian et al 2004; Yalkowsky et al 2006). In this extensive data set, drug products with very high aqueous D_o were completely or near completely absorbed (telmisartan D_o = 660 000; toremifene D_o = 8700; oxatomide D_o = 1500). Only one drug product had a human in-vivo f_a value below 0.8, danazol (D_o = 2400; f_a = 0.30), and nine products had a f_a value below 0.9. D_o data were taken from Yazdanian et al (2004), Kasim et al (2004) and Yalkowsky et al (2006), or calculated from dose and solubility data presented by Willmann et al (2004). F_a data (and P_e data for verifying high P_e) were taken from Irvine et al (1999), Kataoka et al (2003), Zhao et al (2003), Peréz et al (2004), Willmann et al (2004) and Yalkowsky et al (2006). Data by Zhao et al (2001) also demonstrate that substances with very high aqueous D_o are well absorbed: 17 highly permeable compounds (D_o > 20; 12 with $D_o \geq 100$) had f_a values above 0.90. Thus, there is a poor correlation between in-vitro solubility and in-vivo dissolution, only few compounds have dissolution-limited GI uptake, and acceptable extent of GI absorption is achievable for compounds with a D_o up to at least 660 000. This gives support for the proposed solubility/dissolution strictness of the BCS (Yazdanian et al 2004). Furthermore, it indicates that formulation development strategies have been successful. An example of successful formulation development is a 16-fold increase of the oral F for danazol (in the dog) when the particle size was reduced to 85 nm (Liversidge and Cundy 1995).

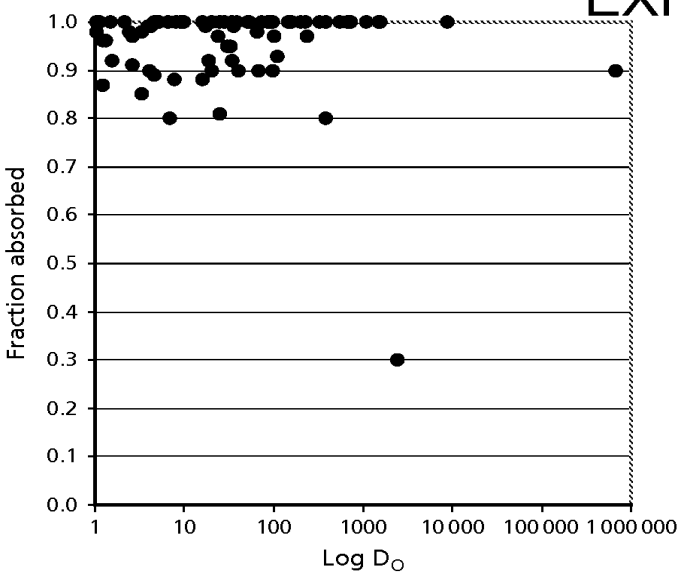

Figure 2 Log in-vitro dose number (D_o) vs in-vivo fraction absorbed (f_a) for high-P_e drug products with low solubility ($D_o > 10$) (n = 73). Despite very high in-vitro D_o values (up to 660 000) many drug products are completely absorbed in-vivo.

D_o data were taken from Kasim et al (2004), Yazdanian et al (2004) and Yalkowsky et al (2006), or calculated from dose and solubility data presented by Willmann et al (2004). F_a data (and P_e data for verifying high P_e) were taken from Irvine et al (1999), Kataoka et al (2003), Zhao et al (2003), Peréz et al (2004), Willmann et al (2004) and Yalkowsky et al (2006). The compound with low f_a is danazol ($D_o = 2400$; $f_a = 0.30$).

A dramatically increased F has been observed for poorly soluble drug products such as danazol ($f_a = 0.30$; $D_o = 2400$), griseofulvin ($f_a = 0.80$; $D_o = 385$) and atovaquone (US Pharmacist website). As an example, a 4-fold increase in oral F of danazol ($D_o = 2400$ in an aqueous medium; $D_o = 400$ in human intestinal fluid; HIF) has been reported after intake with food (Persson et al 2005b). F_a values used in Figure 2 may include data obtained in the fed state (such as for griseofulvin); thus, there may be more highly permeable drug products with dissolution-limited in-vivo uptake than this figure shows.

It is critical that the in-vivo GI fluid environment is mirrored in-vitro. This has been demonstrated by, for example, Patel et al (2005). The extent and rate of in-vitro dissolution of poorly soluble substances in water have been shown to be considerably lower and slower than in SIFs (Galia et al 1998; Yazdanian et al 2004), which further suggests that the use of aqueous media may underestimate in-vivo solubility/dissolution. Takano et al (2006) used solubility in human SIFs, a dissolution parameter simulating in-vivo dissolution, and P_e across the UWL adjacent to the intestinal mucosa to predict the f_a of poorly water-soluble drugs (assuming that permeation across the mucosa is not a rate-limiting step). They found a significant correlation between predicted and observed f_a when dissolution profiles in fasted-state SIF were used for the simulation. Although predictions appear quite good, a drawback with this approach is that diffusion across the UWL does not appear to be a rate-limiting step for in-vivo absorption (Fagerholm and Lennernäs 1995). Data obtained with hydrochloric acid solution and simulated gastric fluid (SGF) are commonly used to predict the in-vivo solubility and dissolution rate in the human stomach (Dressmann et al 1998; Galia et al 1998), and solubility and dissolution data in buffers with different pHs and SIF are often used to estimate the in-vivo dissolution behaviour in the small intestine (Dressmann et al 1998). Optimally, solubility and dissolution should be measured in fresh human GI fluids. Fresh or frozen human gastric fluid and upper small intestinal fluids (HIF) collected during Loc-I-Gut experiments (Lindahl et al (1997) are available for such studies. Persson et al (2005a, b) studied solubility and dissolution rates in fed HIF, fasted HIF and fed SIF for poorly soluble substances, and demonstrated similar dissolution rates in fed HIF and SIF, and 3.5–30-times greater solubility in fed HIF. An alternative could be to use fresh dog GI fluids. Persson et al (2005a) also compared the solubility and dissolution of poorly soluble drugs in human and dog intestinal fluid collected during the fed state and found that, despite differences in composition, the dog appears to be a good model for man with respect to dissolution in the small intestine after food intake. Compounds with very low solubility and slow dissolution rate must, however, rely on dissolution and absorption from the colon, and these processes in this region are difficult to predict.

Actual oral doses of CDs are potentially higher than predicted. This is because of potentials to overestimate f_a from commonly used Caco-2 P_e data (see above) and underestimate clearance (CL) from intrinsic CL (CL_{int}) data. Hepatic CL (calculated from CL_{int}, unbound fraction in blood and hepatic liver blood flow) is underestimated by, on average, 5–9-fold from human microsome CL_{int} and 3–4-fold with human cryopreserved hepatocyte CL_{int} (Iwatsubo et al 1997; Ito et al 1998; Obach 1999; Naritomi et al 2001; Shibata et al 2002; Ito and Houston 2005; Fagerholm 2007b), and extrahepatic CL (which could be important for hydrophilic and metabolically stable compounds) is generally neglected in predictions. These potential underestimations of oral doses could have an impact on the solubility/dissolution, and give erroneous predictions of D_o. The use of allometry is associated with both potential over- and underestimation of CL (Tang and Mayersohn 2006; Fagerholm 2007b). A potential consequence of the underestimation of systemic exposure and dose could be that solubility/dissolution problems are neglected. Overpredictions could lead to exaggerated solubility/dissolution problems.

The approaches presented below take both P_e and solubility/dissolution into account for prediction of f_a. The methods developed by Agoram et al (2001) and Fagerholm and Björnsson (2005) also consider GI degradation.

Maximum absorbable dose (MAD) The MAD approach is often used to predict the absorption potential from P_e and solubility data (Johnson & Swindell 1996). The MAD is the amount of substance that could be absorbed if a saturated solution is absorbed with a first-order rate constant for a time equivalent to the TT of the small intestine (TT_{si}; 3 h). It can be calculated using any of the following equations (Sun et al 2004):

$$MAD = S \times k_{pe} \times V_{si} \times TT_{si} \quad (1)$$

$$MAD = S \times P_e \times 2\pi rL \times TT_{si} \quad (2)$$

where V_{si}, r and L are the human small intestinal volume (usually set to 250 mL), radius (1.75 cm), and length (7 m), and S and k_{pe} are the solubility and permeation rate constant, respectively. $2\pi rL$ is the estimated cylinder area of the small intestine, which, because of the structure of the mucosa, is smaller than the actual area. The k_{pe} can be derived from in-vivo pharmacokinetic data, or the r and measured P_e (e.g. Loc-I-Gut) or predicted P_e of the human small intestine in-vivo (from f_a vs P_e or P_e vs P_e relationships) (Fagerholm et al 1996; Lennernäs et al 1996; Sun et al 2004):

$$k_{pe} = 2P_e / r \quad (3)$$

When k_{ge} is the rate-limiting step for absorption, the k_a can be set to 3.8 h^{-1} (see above), and when dissolution is the rate-limiting step, the k_a can be set to equal the dissolution rate constant (k_{diss}).

Solubility and P_e data for 18 of the 21 compounds presented in Figure 2 were used to calculate the MAD, and these estimates were compared with dose × f_a (which is the lowest possible in-vivo MAD). For seven compounds with incomplete absorption (f_a 0.15–0.99), dose × f_a was 2–160 times (median 4 times) higher than the MAD estimated from equation 1, and varied from 4 times lower to 14-fold higher (median 1.6 times higher) than the MAD estimated from equation 2. The corresponding values for the 11 completely absorbed substances were 3–84 times higher (median 23 times), and varied from 20% lower to 84 times higher (median 5 times higher), respectively. Inclusion of the correction factor for adjusting TT_{si} to the effective intestinal TT (f = 2.8; see below; Fagerholm et al 1996) produced smaller differences. In-vivo MAD data are missing and these could differ widely from dose levels at which saturation begins. An AstraZeneca compound under development that has a predicted MAD of 5–10 mg had still not reached the MAD in animals at dose equivalents above 6 g. This further demonstrates the potential of MAD to underestimate (probably by orders of magnitude) and the poor relationship between in-vitro solubility and in-vivo dissolution.

Advanced compartmental absorption and transit (ACAT) model Yu et al (1996a) developed a compartmental absorption and transit (CAT) model to simulate the GI absorption for compounds with P_e-limited uptake. This model assumes minor gastric and colonic absorption, linear small intestinal uptake and instantaneous dissolution, and divides the small intestine into seven different compartments (which seemed to give a slightly better fit to observed TT data than five or nine compartments (Yu et al 1996b)). The advanced CAT (ACAT) is a further development of the CAT model, and is the basis of the software package GastroPlus (Agoram et al 2001). The ACAT model includes compartments for stomach and colon, and enables prediction of the absorption of slowly and incompletely dissolved compounds/dosage forms, and for substances degraded in the GI contents (Agoram et al 2001). The model accounts for pH dependency of dissolution and P_e. Parrott and Lavé (2002) used this method together with Caco-2 P_e and solubility data, and found comparably poor predictions of f_a (see above). There was a tendency towards overestimation, which might lead to selection of CDs that fail to reach desired exposure profiles. According to D_o data, the main reason for poor predictions is probably the comparably weak predictability of the Caco-2 model. None of the compounds appeared to have solubility/dissolution-limited GI absorption. In order for GastroPlus and other programs to predict f_a well, the in-vitro P_e and solubility/dissolution must mirror the in-vivo situation. As shown above, Caco-2 P_e and aqueous solubility/dissolution data do not appear sufficiently good. For optimal performance, data obtained with the most suitable P_e and dissolution models should be used, and P_e and dissolution in various GI regions (such as the eight intestinal compartments in GastroPlus) must be considered and well predicted. As discussed above, very few compounds appear to have dissolution-limited GI absorption. Therefore, P_e-based methods would probably be sufficient to predict f_a in most cases.

One-compartmental absorption and transit (OCAT) model Fagerholm et al (1996) used Equation 4 to establish the relationship between human small intestinal in-vivo P_e (obtained with Loc-I-Gut) and f_a for soluble compounds. TT_{si} and r were set to 3 h and 1.75 cm, respectively. The correction factor (f; estimated to 2.8) adjusts for differences between the chosen and effective TT_{si}, r and absorption area, and spreading of the dose along the GI canal (Equation 5). Thus, with this factor, colonic absorption is taken into account and there is no requirement to divide the intestine into several compartments (as in ACAT). The total effective time for dissolution and absorption is estimated to be 8.4 h (3 h × 2.8).

$$f_a = 1 - e^{-2\,(Pe \times TTsi \times f)\,/\,r} \quad (4)$$

$$f_a = 1 - e^{-2 \times Pe \times 3\,h \times 2.8\,/\,1.75\,cm} \quad (5)$$

For a compound with a k_{diss} that is slower than k_{pe}, $2 \times P_e \times 3\,h \times 2.8 / 1.75$ cm can be replaced by $k_{diss} \times 3\,h \times 2.8$.

Fagerholm and Björnsson (2005) used this OCAT model to predict the f_a of the highly lipophilic ester pro-drug AZD3582 and its active metabolite naproxen. AZD3582 is unstable in intestinal fluid, and a self-emulsifying delivery system was required to provide the desired GI dissolution and absorption. Its P_e measured in human small intestine in-vitro using the Ussing chamber was moderate to high, and 1/40 of that measured for the highly permeable naproxen. P_e in human small intestine in-vivo was estimated from the relationship between in-vitro and in-vivo small intestinal P_e. Rate constants for dissolution in SGF (k_{diss}), degradation in HIF (k_{degr}) and permeation (k_{pe}; using Equation 3) for AZD3582 were estimated to be 1.4, 0.23 and 0.08 h^{-1}, respectively. The k_{pe} for naproxen was estimated to be 3.2 h^{-1}, and the k_{ge} was set to 3.8 h^{-1} (see above). Thus, gastric emptying is not a rate-limiting step, AZD3582 is more rapidly (and completely) dissolved than it is degraded and absorbed, and degradation of AZD3582 is 3 times more rapid than permeation. The f_a of intact AZD3582 (f_a*) was estimated using Equations 5 (to estimate the f_a if AZD3582 were stable) and 6.

$$f_a^* = f_a \times k_{pe} / (k_{pe} + k_{degr}) \quad (6)$$

The f_a* of AZD3582 after oral dosing was predicted to be 0.23–0.24. Intravenous pharmacokinetic data were lacking, and therefore it was not possible to validate the prediction of AZD3582 uptake. In-vivo pharmacokinetic and mass-balance data indicated, however, that AZD3582 must have been absorbed to at least 9–20% as intact substance. The f_a and k_a for naproxen after AZD3582 and naproxen administration were well predicted.

New approach for prediction of gastrointestinal absorption from permeability

Highly soluble compounds with high P_e are generally completely absorbed from the small intestine, whereas compounds with low/moderate small intestinal P_e and slow/incomplete dissolution must rely on uptake from both the small and large intestines. As described above, the P_e characteristics differ between the small and large intestines, and there is a high correlation between small intestinal absorption and P_e in humans and rats. On this basis, it is reasonable to assume that f_a values for incompletely absorbed compounds lie somewhere between estimates generated with the 2/4/A1 and Caco-2 cell lines. A comparison of f_a data obtained with these models (taken from Matsson et al 2005), and the average of 2/4/A1 and Caco-2-data for 12 passively transported compounds is shown in Figure 1. The 2/4/A1 cells appear to give best predictions when f_a is above 0.9 (underestimated with Caco-2), and 2/4/A1 cells and the combined average approach seem to perform similarly and best when f_a is below 0.9. The average (median, maximum) relative prediction error for 2/4/A1 cells, Caco-2 cells and the combined approach for compounds with f_a above 0.9 is 2% (2, 8), 10% (10, 13) and 6% (6, 9) respectively. The corresponding numbers for compounds with f_a of 0.25–0.9 are 14% (13, 22), 27% (27, 53) and 15% (16, 24), respectively. The 2/4/A1 data slightly overestimated the f_a of terbutaline (log D −1.4, f_a 0.60), whereas Caco-2 cells underestimated it to a considerable extent. All methods overestimated the f_a of two compounds with very low f_a. The greatest overestimation (6 fold) for these compounds was found with Caco-2 cells. The 2/4/A1 cells gave the lowest overestimation (3–4 fold). Thus, Caco-2 cells have the greatest potential to give an incorrect indication that a compound that has unfavourable absorption characteristics will be sufficiently well absorbed in-vivo, and to misclassify high-P_e compounds (according to the BCS). The results obtained with this limited data set also show that, except for one intermediately permeable compound (terbutaline), the combined approach does not perform better than the 2/4/A1 method. This could be an indication that incompletely absorbed soluble drugs (at least the ones in the study) are mainly taken up by the small intestine.

Conclusion

The most accurate P_e models for prediction of f_a of passively transported and highly soluble compounds appear to be the rat small intestinal 2/4/A1 cell model (in-vitro and in-silico), a newly developed artificial membrane method and a semi-empirical approach based on in-vitro membrane affinity to immobilized lipid bilayers, effective MW and physiological GI variables. The limited amount of data from the 2/4/A1 model demonstrates a potential to obtain appropriate f_a predictions, CD selection and BCS classification. This method also appears to be technically demanding. Additional experiments are needed to fully evaluate the potential of this model. Good f_a predictions and CD selection is also achievable with the semi-empirical approach. However, this method does not enable correct BCS classification for compounds with near-complete to complete f_a. The predictability of in-vitro Caco-2 cells, in-situ perfusion and other artificial membrane methods seems comparably low (high potential for incorrect CD selection/rejection and BCS classification). The average of P_e values obtained from the 2/4/A1 model (rat small intestinal cells) and Caco-2 (human colonic) cells, which is a new proposed approach, appears better than with Caco-2 cells, but does not appear to give better predictions than the 2/4/A1 model alone. This suggests that these incompletely absorbed soluble drugs are mainly taken up by the small intestine. The methods evaluated generally work well to predict complete or near complete f_a for compounds with high passive P_e. Values of P_e and f_a in humans for compounds with mainly active transport are poorly predicted with all models investigated (except when the passive P_e is high). However, the rat in-situ perfusion and Caco-2 models appear useful for prediction of active uptake potential (complete active uptake is well predicted) and studies of bidirectional active transport, respectively. Human intestinal in-vitro P_e, which correlates well with f_a for passively transported compounds, could possibly also have potential to improve/enable f_a predictions of actively transported substances.

The 'rule-of-five', modified 'rule-of-five', PSA and other in-silico approaches could give an indication of the absorption potential. Good permeation potential is generally anticipated if PSA is below 140 $Å^2$ and MW is below 500 g $mole^{-1}$, and good dissolution potential can be expected if log P is below 5. Good in-vivo uptake has, however, been demonstrated for compounds with considerably larger estimates than these.

The MAD and D_o approaches, and solubility/dissolution data obtained in aqueous media, appear to underestimate in-vivo dissolution to a considerable extent. For most highly permeable compounds with low in-vitro solubility, the extent of in-vivo dissolution does not seem to influence the f_a. For highly permeable compounds with low solubility (BCS class II) only one with f_a below 0.8 and nine with f_a below 0.9 (out of 73 low-solubility drug products) have been found. Complete or near-complete uptake has been found for substances with a D_o up to 660 000. On the basis of these findings, it appears that, in most cases, the f_a could be predicted from P_e only. In order to predict D_o well before the start of clinical trials, P_e, metabolism and doses need to be predicted accurately. Commonly used methods for prediction of these are, or could be, misleading, and the consequences are exaggerated or neglected solubility problems. Prediction of in-vivo dissolution should preferably be done from in-vitro dissolution data obtained using real, or validated simulated, GI fluids.

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